

REMARKS

Claims 1-19 are pending in the present application. Claims 8-19 are withdrawn as directed to a non-elected invention. It is respectfully requested that the Examiner reconsider the rejection of the claims 1-7 in view of the following remarks.

Claim Rejections-35 U.S.C. § 101

Claims 1-7 are rejected under 35 U.S.C. § 101 as lacking patentable utility. The Examiner states that claims 1-7 read on a product of nature "because these truncated glucanases can exist in nature." We respectfully traverse. The present invention provides truncated glucanases that is about 10 kDa smaller than the full length wild-type enzyme that is found in nature (see page 4, lines 14-17 of the present application). The truncated glucanases exhibit significantly higher specific activity compared to the full length wild-type enzyme (see page 5, lines 1-20), and are able to maintain their activity at high temperature (see page 6, lines 1-9). Claims 1-7 are directed to these novel truncated glucanases. Such truncated glucanases are therefore distinguishable from products of nature. Accordingly, it is respectfully requested that the rejection of claims 1-7 under 35 U.S.C. § 101 as lacking patentable utility be withdrawn.

Claim Rejections-35 USC § 112

Claims 1-2 are rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter not described in the specification as to convey to one of ordinary skill in the art that the inventors had possession of the claimed invention at the time the application was filed. On page 4 the Examiner states "[c]laims 1-2 are rejected under 35 U.S.C. 112, first paragraph,

Serial No.: 09/654,652

because the specification, while being enabling for the truncated 1,3-1,4-beta-D-glucan 4-glucanohydrolases from *F. succinogenes* of SEQ ID NO:3, does not reasonably provide enablement for any truncated family of glucanases from *F. succinogenes*."

The Examiner also stated that the "specification fails to provide guidance regarding how to make truncated glucanases from any glucanases from *F. succinogenes* 1,3-1,4- $\beta$ -D-glucanase." (page 4 of the Office Action) In response, claim 1 has been amended to clearly recite the N-terminal amino acid residues that are claimed.

There is presently only one known wild-type form of *F. succinogenes* 1,3-1,4- $\beta$ -D-glucanase enzyme. Contrary to the Examiner's statement, there are no other glucanase enzymes of *F. succinogenes* from which one can derive the presently claimed truncated forms of 1,3-1,4- $\beta$ -D-glucanase. Therefore, the specification in present patent application is enabling and does disclose the identifying characteristics that can allow one of ordinary skill in the art to recognize the truncated forms of 1,3-1,4- $\beta$ -D-glucanase from *F. succinogenes*, as recited in claims 1 and 2. The present application teaches that the C terminal truncation is done by use of a specific and defined molecular cloning method via PCR priming. Claims 1-2 are enabled by the following disclosure in the specification.

In the present patent application on pages 11-18, it is clearly disclosed the specific and reproducible molecular manipulations (e.g., using specific primer design and PCR techniques) for the creation of the N-terminal truncated forms of 1,3-1,4- $\beta$ -D-glucanase from *F. succinogenes*. The inventors have fully characterized the catalytic and thermostability properties of the enzymes generated in this specific way. The results are presented in Figures 4 and 5 and Tables 1 and 2 in the present application. The present invention is simple, clear cut, intelligent and it works.

The present application demonstrates that an appropriate C terminal truncation of the wild-type 1,3-1,4- $\beta$ -D-glucanase from *F. succinogenes* can create specific, shorten molecular forms of mutant enzymes which can confer a significant increase in their specific activities and thermal stability as compared to the parental wild-type enzyme. The two truncated forms of glucanases that are encompassed by claims 1 and 2 (differing only in the process of producing them) are both derived from the same parental wild-type enzyme. The two enzyme sequences of the present invention SEQ ID NO 1 and 2 of the contain 248 and 267 amino acid residues, respectively. These two truncated glucanases show very similar kinetic properties even though they have difference of a nineteen amino acid residues in the molecular size of the protein. The present application has further shown the "range or flexibility" of the enzyme truncation process.

Accordingly, it is respectfully requested that the rejection of claims 1-2 under 35 U.S.C. § 112, first paragraph, be withdrawn.

Claims 1-7 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite. The Examiner states that "[I]t is unclear which amino acid sequences are encompassed by the claim." Specifically, the Examiner states that the term "substantially identical" is unclear. We respectfully traverse. The present application on page 10, line 19 to page 11, line 2 states that the term is meant to cover enzymes or DNA coding fragments that have minor sequence variations from the specified sequences that do not effect the enzyme's enzymatic function to any significant degree. Thus, the term "substantially identical" is related to enzymatic function, and its use does not render claims 1-7 indefinite.

Accordingly, it is respectfully requested that the rejection of claims 1-7 under 35 U.S.C. § 112, second paragraph, as being indefinite be withdrawn.

Claim Rejections-35 USC §102

Claims 1-3 and 6-7 are rejected under 35 USC §102(e) as being anticipated by U.S. Patent 6,103,511 of Li et al. (the Li Patent).

The Examiner states that "Li et al Patent (No. 6,103,511) describes a truncated glucanase of at least 200 amino acid residues forming a linear sequence to the amino acid sequence of a wild-type glucanase from *Fibrobacter succinogenes*, SEQ ID NO:3 of the instant invention, and contains no PXSSSS repeats. The truncated glucanase of Li et al. is also substantially identical to SEQ ID NO:1 and 2 of the instant invention. Therefore the glucanase of Li et al. anticipates claims 1-3 and 6-7." We respectfully traverse.

The Li Patent is directed to the amino acid and DNA coding sequences of a fungal lichenase (1,3-1,4- $\beta$ -D-glucanase) isolated from *Orpinomyces* PC-2. The Li Patent is also directed to an identical or a functionally equivalent sequence, either in a form of DNA or amino acid sequence, with at least about 70% identity to the sequences of the fungal lichenase.

The following outlines the difference between the Li Patent and the presently claimed invention.

1. The truncated 1,3-1,4- $\beta$ -D-glucanase DNAs/enzymes of the present invention are derived for a bacterial source: *Fibrobacter succinogenes*. In contrast, Li et al isolated their DNA/enzyme from a fungal source: *Orpinomyces* PC-2.

2. The protein sequence homology of the truncated forms of *F. succinogenes* 1,3-1,4- $\beta$ -D-glucanase, as described in the present application, are only 30-35% identical to that of the *Orpinomyces* PC-2 enzyme of the Li patent, as analyzed by a Genetics Computer Group, Inc. (Madison, WI) in the present inventors' studies.

3. The *Orpinomyces* PC-2 lichenase of the Li et al patent is a full-length native form enzyme with or without the N-terminal leader sequence. In the absence of N-terminal leader sequence, which usually is taken away from the enzyme by the cleavage of signal peptidase during the protein expression process, the *Orpinomyces* PC-2 lichenase consists of 216 amino acid residues as recited in the claims of the Li Patent. In contrast, the presently claimed enzymes are recombinant and created using definable molecular biological techniques from the wild-type *F. succinogenes* 1,3-1,4- $\beta$ -D-glucanase.

4. The presently claimed enzymes (truncated 1,3-1,4- $\beta$ -D-glucanase from *Fibrobacter succinogenes*) possess a high specific activity (7,833 U/mg), which is 2-fold higher than that of *Orpinomyces* PC-2 lichenase (3,786 U/mg).

5. The present inventors have provided a comparison of kinetic properties of various bacterial and fungal 1,3-1,4- $\beta$ -D-glucanases (see attached Declaration).

6. The presently claimed glucanase can recover 80 to 85% of its original enzymatic activity after intense heat denaturation (10 min at 90 °C) and approximate 50 % activity after boiling (see Figure 5 in the present application). The enzyme disclosed in the Li Patent does not possess the temperature-resistant properties of the presently claimed enzymes.

In short, there is a) very low (~30%) amino acid sequence homology between the enzymes disclosed in the Li Patent and those presently claimed; b) the Li Patent does not disclose any effect of enzyme function or activity by C-terminal truncation or deletion on enzymes from any bacterial, fungal, or any other organisms; and c) there is no correlation (based on structure or sequence) between the "shorter" enzymes disclosed in the Li Patent and the presently claimed truncated glucanases. The molecular mass of the presently claimed two truncated enzymes are 27744 and 29722, as determined by mass spectrometry. In contrast, the

Serial No.: 09/654,652

enzyme disclosed in the Li Patent is 27 kDa as determined by SDS-polyacrylamide gel electrophoresis.

Accordingly, it is respectfully submitted that the rejection of claims 1-3 and 6-7 under 35 USC §102(e) as being anticipated by the Li Patent be withdrawn.

It is respectfully submitted that the claims are in a condition for allowance, early notice of which is earnestly requested.

It is believed that no fees or charges are required at this time in connection with the present application; however, if any fees or charges are required at this time, they may be charged to our Patent and Trademark Office Deposit Account No. 03-2412.

Respectfully submitted,  
COHEN, PONTANI, LIEBERMAN & PAVANE

By Kent H. Cheng  
Kent H. Cheng  
Reg. No. 33,849  
551 Fifth Avenue, Suite 1210  
New York, New York 10176  
(212) 687-2770

Dated: April 19, 2002



Version with Changes to Show Markings in Claim(s)

1. (Amended) A truncated glucanase having an amino acid sequence with a total number of N-terminal amino acid residues between 200 and 321, wherein at least 200 of said amino acid residues [forming] form a linear sequence substantially identical to a portion of the N-terminal amino acid sequence of a wild-type glucanase from *Fibrobacter succinogenes*.